成長板軟骨細胞におけるTRPM7チャネルを介する自発的Ca2+変動(Abstract_要旨)

AUTHOR(S):

銭, 年超

京都大学	博　士　　（ 薬科学 ）	氏 名	銭　年超
論文題目	Spontaneous Ca^{2+} fluctuations mediated by TRPM7 channels in growth plate chondrocytes（成長板軟骨細胞におけるTRPM7チャネルを介する自発的Ca2+の変動）		

（論文内容の要旨）

During embryonic bone development, round chondrocytes proliferate, differentiate into columnar chondrocytes, and then further differentiate into hypertrophic chondrocytes in the growth plate chondrocytes. Studies over the past few decades have identified a multitude of signaling and transcription factors that regulate these progressive changes in chondrocytes.

Intracellular Ca^{2+} signaling plays fundamental roles in cellular homeostasis and may control some processes during the chondrogenesis. However, Ca^{2+}-handling at different chondrocyte developmental stages is virtually uncharacterized. Therefore, I attempted to clarify the Ca^{2+}-handling features of chondrocytes and address the physiological role of Ca^{2+} signaling in bone development.

Chapter1. Spontaneous Ca^{2+} fluctuations in growth plate chondrocytes

I developed a method to prepare sliced cartilages from embryonic day 17.5 mouse embryos to measure intracellular Ca^{2+} signaling in intact chondrocytes. Approximately 20% and 10% of round and columnar chondrocytes exhibited spontaneous changes in intracellular calcium concentration (spontaneous Ca^{2+} fluctuation). The Ca^{2+} fluctuations were abolished by Ca^{2+} removal and non-specific Ca^{2+} channel inhibitor Gd^{3+} in round chondrocytes, whereas activated by the sarco/endoplasmic reticulum Ca^{2+}-pump inhibitors, thapsigargin and cyclopiazonic acid (CPA). The results indicated that the Ca^{2+} fluctuations were generated by Ca^{2+} influx.

To identify Ca^{2+} channels responsible for generating the Ca^{2+} fluctuations on plasma membrane, microarray analysis was conducted using RNA which prepared from the region enriched with round chondrocytes and the region containing columnar and hypertrophic chondrocytes. Transient receptor potential (TRP) channel family genes (*Trpm4*, *Trpm5*, *Trpm7*, *Trpc1*, *Trpc2* and *Trpv4*), Purinergic P2X (P2X4, P2X7), voltage-gated Ca^{2+} channels, ORAI channels (*Orai1*, *Orai2* and *Orai3*), were highly expressed in the two regions. Reported inhibitors of these Ca^{2+} channels on plasma membrane were tried. However, only inhibitors of TRPM7, FTY720 and NS8593, exerted obvious effects on the Ca^{2+} fluctuations. The Ca^{2+} fluctuations were attenuated by the two inhibitors. In addition, the reported TRPM7 activators naltriben and NNC550396 facilitated the Ca^{2+} fluctuations.

To confirm the effect of TRPM7 modulators, *Trpm7*-deficient bones were analyzed in this research. The percentage of the Ca^{2+} fluctuations-positive cells was reduced. Amplitudes of the Ca^{2+} fluctuations were dampened under both basal and naltriben-treated conditions in *Trpm7*-deficient chondrocytes. Phospholipase C (PLC) and big-conductance Ca^{2+}-activated K^+ (BK) channels have been reported to cross-talk with TRP channels. In this study, PLC inhibitor U73122 and BK channel opener NS1619 were applied to bone slices. U73122 effectively attenuated the Ca^{2+} fluctuations under normal and naltriben-treated conditions, while its inactive homologue U73343 had no significant effects. NS1619 markedly facilitated the Ca^{2+} fluctuations under basal and naltriben-treated conditions. Furthermore, FTY720 pretreatment could abolish NS1619-treated effects and the BK channel inhibitor paxilline could clearly attenuate naltriben-treated effects.

Based on the results above, spontaneous Ca^{2+} fluctuations were mediated by TRPM7 channels and maintained by PLC and BK channels in growth plate chondrocytes.

Chapter2. Role of the spontaneous Ca^{2+} fluctuations mediated by TRPM7 in bone development

Metatarsal organ culture is widely utilized as a model system for studying endochondral bone development. To examine the physiological role of the Ca^{2+} fluctuations mediated by TRPM7 channels, the third *Trpm7*-deficient metatarsals were cultured for 8 days. The *Trpm7*-deficient bones exhibited outgrowth retardation accompanied by diaphyseal reduction.

Histological analysis showed that the total epiphyseal area of *Trpm7*-deficient bones was reduced, which was consistent with their outgrowth retardation. When the epiphyseal region was divided according to their characteristic morphological features, the area of columnar and hypertrophic zones were reduced, and columnar zone showed more obvious reduction rate than hypertrophic zone, even though round zone maintained the regular area. Furthermore, hypertrophic chondrocytes became smaller in *Trpm7*-deficient bones, while both round and columnar chondrocytes preserved normal size. Given to the results above, maturation processes of growth plate chondrocytes seem to be controlled by TRPM7 channels in bone development.

In conclusion, coupled with phosphatidylinositol turnover and plasma membrane K^+ efflux, TRPM7 channels mediated spontaneous Ca^{2+} fluctuations in growth plate chondrocytes and played essential roles in cellular maturation toward bone development. The present study is expected to have implications for prevention and treatment of joint diseases in the orthopedic field.

（続紙　２　）

（論文審査の結果の要旨）

本提出論文に記述された研究により、骨成長板を構成する軟骨細胞はTRPM7チャネルによる自発性$Ca2^{+}$振幅を発生することが見出されるとともに、その$Ca2^{+}$流入は軟骨細胞の増殖・分化を促進することで骨成長に寄与することも明らかにされた。この成果は基礎生物学の進展に貢献するとともに、薬学領域にも重要な波及効果を有する。よって、本論文は博士（薬科学）の学位論文として価値あるものである。

また、平成30年2月23日、論文内容とそれに関連した事項について試問を行った結果、合格と認められた。

要旨公表可能日：平成３０年６月２５日以降